Report for 2005VI51B: Qualification and quantification of human bacterial pathogens in UVI aquaculture systems

Publications

- There are no reported publications resulting from this project.

Report Follows

Problem and Research Objectives

The aquaculture program of the University of the Virgin Islands' Agricultural Experiment Station has developed aquaculture systems which are being adopted by farmers in the U. S. Virgin Islands, the USA and in other countries. As implementation of these systems proceeds in these locations, health and environmental monitoring agencies involved in the permitting process have requested information on the presence of human bacterial pathogens in the aquaculture systems' water and the potential for illness among workers and consumers and the environmental impact of waste water discharged.

Coliform bacteria are a group of bacteria of the family *Enterobacteriaceae* "consisting of gram negative aerobic and facultatively anaerobic rods which produce acid from glucose and other carbohydrates, and are usually aerogenic" (Speck 1976). They are ubiquitous in the intestinal track of animals and in soil, plants and water. Fecal coliform is a subset of total coliform and are found in the digestive track of warm-blooded animals and birds. The presence of fecal coliform in water is not in itself a hazard but its identification in water is used as an indicator of possible contamination by other pathogenic bacteria. *Escherichia coli* is one specie of fecal coliform that is monitored in public drinking and recreational water. *E. coli* O157:H7 is a strain of these bacteria that does produce a toxin that causes severe illness in humans and is a public health concern that is monitored by Territorial and Federal agencies. There were 183 reported cases of Shinga toxin-producing *E. coli* in the United States in 2005. There were no reported cases in the U.S. Virgin Islands (MMWR vol. 55:8). Not all cases of gastroenteritis are reported to public health officials and the cause of each case is often unknown

The UVI aquaculture systems studied in this research are exposed in an outdoor environment and accessible by birds and mammals. Feces from these animals can contaminate the water with coliform and other pathogenic bacteria. This research evaluated water samples from the systems over a 6-month period to determine the presence of coliform, fecal coliform and reported the number of organisms in a 100 mL sample.

Methodology

Water samples were collected from the UVI Aquaponic System, Greenwater Tank Culture System, and the Fish Purge system during the Summer, Fall and Winter, 2005-2006. Samples were collected in sterile plastic bottles at a depth of 30 cm from the surface. They were immediately placed in an ice chest and transported to a commercial water quality analysis laboratory for analysis.

Using Standard Method 9221 B. Standard Total Coliform Fermentation Technique (Eaton, 1995) the laboratory performed the presumptive portion of the multiple-tube test. This test uses 9 tubes of lauryl tryptose broth, each of which is inoculated with a different

dilution of aquaculture system water. These tubes are incubated for 24 ± 2 hr at 35 ± 0.5C. They are examined for growth, gas, and acidic reaction and the results recorded.

The Estimation of Bacterial Density, Standard Method 9221 C, was made for positive results from the Coliform Fermentation Technique. The results of these methods are included in Table 1.

Table 1. Total coliform counts, MPN reported organisms per 100 mL,
for three aquaculture systems.

Date	29-Jun-05	12-Jul-05	30-Aug-05	3-Nov-05	29-Nov-05	13-Dec-05	10-Jan-06	24-Jan-06	7-Feb-06
Aquaponic	tntc	2,300	300	3,000	50,000,000	3,000	800	2,300	2,300
Greenwater	929	230,000	1,100,000	1,700	1,600	2,300	5,000	300,000	900
Purge	35	800	110,000	11,000	2,800	230		500,000	5,000

Fecal Coliform Procedure 9221 E was used to determine the presence and number of fecal coliform. This elevated-temperature test is conducted after Total Coliform Technique has confirmed the presence of coliforms. The Estimation of Bacterial Density, Standard Method 9221 C, determines the Most Probable Number (MPN) reported organisms in a 100 mL sample. The results of this method are included in Table 2.

Table 2. Fecal coliform counts, MPN reported organisms per 100 mL,
for three aquaculture systems.

Date	29-Jun-05	12-Jul-05	30-Aug-05	3-Nov-05	29-Nov-05	13-Dec-05	10-Jan-06	24-Jan-06	7-Feb-06
Aquaponic	230	1,100	230	230	23	23	23	30	
Greenwater	230	170,000	230	23	230	300	23	50	
Purge		23	13,000	2,300	2	50		23,000	300

Two sections of the UVI course Science 200 "Changes in the Natural World" were taught. Each laboratory section had 15 students who took the course in partial fulfillment of the general education requirements of the BA degree. A presentation was made describing bacterial pathogens in general, the research undertaken and results. The students then learned to use 3M Petrifilm™ plates for the identification and enumeration of Coliform and E. coli. These are gel plates that are inoculated with a measured amount of solution and then incubated for 24 – 48 hrs. to determine the presence of coliform by color change of the gel and production of gas bubbles under the cover film. The students learned laboratory quality control, pipetting techniques, and identification and counting strategies. At the close of one class a student remarked, "You guys make learning fun!"

Principal Findings and Significance

Water sample analysis on each date and each system found both total and fecal coliform counts in measurable numbers. On the first sample date the total coliform in the Aquaponic System was "too numerous to count." After this date the laboratory included

a dilution range that encompassed high values. In one instance the MPN of total coliform was 50,000,000. Excluding this high point the next highest MPN for total coliform in the Aquaponic System was 3,000 organisms per 100 mL (Table 1.) Fecal coliform ranged from 23 to 1,100 organisms per 100 mL (Table 2.)

The Greenwater System had a wide and inconsistent range of total coliform, from low of 900 to high 1,100,000 MPN organisms per 100 mL and fecal coliform from low 23 to 170,000 MPN organisms per 100 mL. No pattern of increasing or decreasing numbers of organisms could be determined from the sample results.

The Purge System had a wide range of organisms reported, both total coliform and fecal coliform were present. The range of total coliform was 230 to 500,000 MPN organisms per 100 mL. The range of fecal coliform was 2 to 23,000 MPN organisms per 100 mL.

Coliforms should be considered part of the normal flora of an aquaculture system. They are ubiquitous in the environment and cannot be eliminated. The UVI aquaculture systems are outdoors and open to wildlife and domestic animal contact. Changing this condition would require extensive modification of the system and change the essential nature of the systems. Farmers could set up fencing and nets to reduce intrusions but contact with coliforms is unlikely to be eliminated.

High levels of these indicator bacteria have little or no influence on the quality of fish for human consumption. While alive, the fish is protected from waterborne contaminants by the mucus, scales and skin covering its body. Proper fish cleaning, rinsing, refrigeration and cooking should always be used. This will eliminate the pathogenic bacteria from the food. The implementation of a Hazard Analysis Critical Control Point (HACCP) plan at a fish processing facility will guard the safety of food fish from the farm to the point of sale.

The public health risk from eating tilapia produced and sold from UVI designed aquaculture systems cannot be determined from this research. Epidemiological studies that trace illness and disease back to their point of origin are needed to determine if and when coliforms present in aquaculture systems cause disease. The USVI Department of Public Health had no cases reported cases of illness caused by E. coli in the 52 week period ending March 3, 2006 (MWWR 2006. 55:08 p.223).

References

Eaton, A.D., L.S. Clesceri, and A.E. Greenberg. eds. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. American Public Health Association. Washington, DC.

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Speck, M.L. ed. 1976. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association. Washington, DC.